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Identification of the 22nd Genetically-encoded Amino Acid in a Methanogen Methyltransferase

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Proteins have long been known to be composed of only 20 building blocks, called amino acids. But about 25 years ago, scientists discovered an additional, 21st amino acid, called selenocysteine. Now, two groups of researchers led by biochemist Michael Chan and microbiologist Joseph Krzycki, both of The Ohio State University in Columbus, have identified the 22nd amino acid in an enzyme, called methyltransferase, which breaks down methylamine (CH_3NH_2) in methane(CH_4)-producing microbes called methanogens, leading to the production of methane. The scientists call this new amino acid pyrrolysine.

Inside cells, proteins are synthesized by two processes, called transcription and translation. In the first process, the genetic information of DNA is "transcribed" into messenger RNA (mRNA). In the second process, mRNA is "translated" into a series of amino acids, the building blocks of proteins, which self-assemble to form the protein.

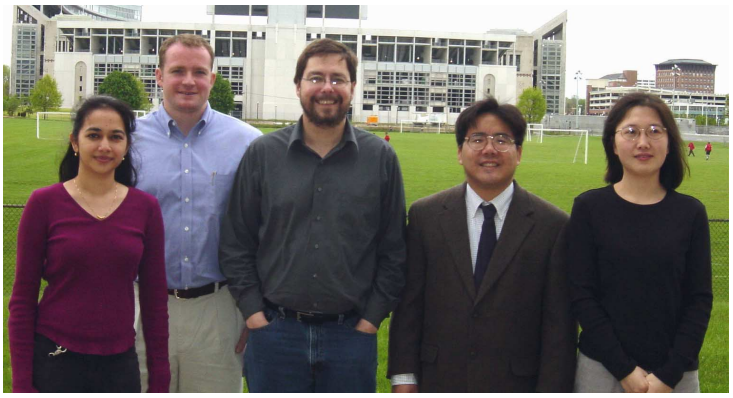
During the translation process, a protein/RNA complex called the ribosome attaches to the mRNA, and reads its nucleotides (uracil U, adenine A, guanine G and cytosine C) three by three. Inside the ribosome, transfer RNA (tRNA) molecules attach to each set of three nucleotides, or codon, and provide the corresponding amino acid, which is added to a chain of amino acids that folds and creates, little by little, a protein.

Each of the 64 possible codons has a specific tRNA that recruits one of 20 "standard" amino acids, except for UAA UAG and UGA. These three codons are generally used to indicate the end of the amino acid chain. In certain cases, however, a specific tRNA associates UGA to a "nonstandard" amino acid, the 21st amino acid, called selenocysteine.

Over the last few years, two groups of scientists at The Ohio State University in Columbus, led by biochemist Michael Chan and microbiologist Joseph Krzycki, initiated a collaboration to further identify what amino acid is encoded by UAG.

The Krzycki group found that a microbe known to produce methane (CH_4), called *Methanosarcina barkeri*, contain a UAG-decoding tRNA (figure 1), and a unique lysyl-tRNA synthetase (PylS), a protein that helps translating the UAG codon. Krzycki and his colleagues noticed that PylS could charge the UAG-decoding tRNA with lysine, which is then enzymatically modified to form another amino acid.

To characterize the identity of the UAG-encoded amino acid, the structure of the monomethylamine methyltransferase was determined by the Chan group. Two forms of the enzyme were obtained from crystallization conditions that differed only in the precipating salt used [NaCl and $(NH_4)_2SO_4$] and were solved to 1.55 angstrom (Å) and 1.7 Å resolution, respectively.



Members of the two teams that conducted the study (from left to right): Gayatrhi Srinivasan, Carey M. James, Joseph A. Krzycki, Michael K. Chan, Bing Hao.

The 1.55 Å resolution methyltransferase structure reveals a hexameric protein, with each subunit adopting a TIM barrel fold – a structure present in 15 enzyme families (figure 2). The UAG-encoded amino acid, called pyrrolysine, lies at the bottom of a cleft,

shown as a ball-and-stick model in figure 2.

Fitting of the electron density from the two different crystal forms suggests that the UAG encoded amino acid is described by the chemical formula: 4-substituted-(4R,5R)-pyrroline-5-carboxylate, with the carboxylate of the modifying group attached to the epsilon nitrogen of lysine (figure 3).

By showing that pyrrolysine corresponds to a UAG codon in some genes, and that an amber decoding tRNA is found in organisms containing these genes, the researchers demonstrate that pyrrolysine is the 22nd genetically encoded amino acid to be identified in nature.

For more details...Srinivasan, G., C. James, and J. Krzycki "Pyrrolysine Encoded by UAG in Archaea: Charging of a UAG-Decoding Specialized tRNA", Science, **2002**, 1459-1462.

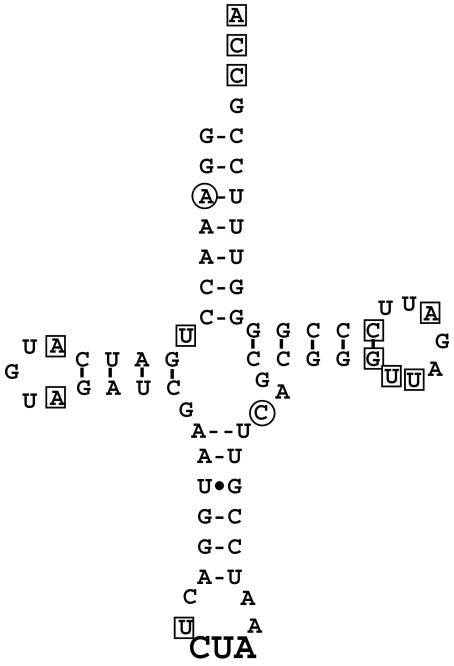


Figure 1. *Structure of the UAG-decoding tRNA*

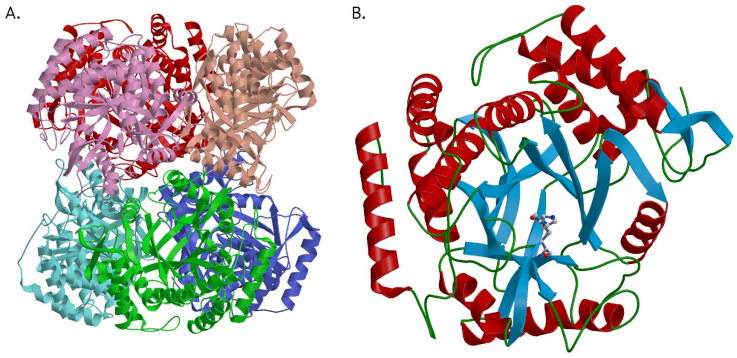


Figure 2. *Ribbon diagram of a subunit of the Methanosarcina barkeri monomethylamine methyltransferase hexamer (α helices: green; ß sheets: cyan; random coil: yellow). The atoms of the UAG-encoded residue are shown as ball-and-stick models and are colored by their elements, with carbon as gray, nitrogen as blue, and oxygen as red.*

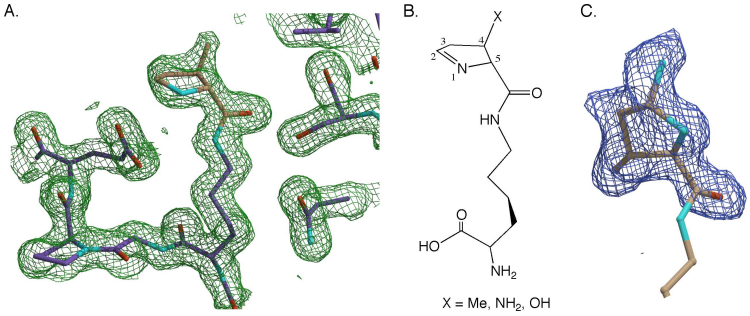


Figure 3. (A) *Fit of 4-substituted-(4R,5R)-pyrroline-5-carboxylate to the electron density of the NaCl crystal form of pyrrolysine.* ***(B)*** *Stick-diagram of proposed pyrrolysine amino acid. The substituent attached to the C-4 carbon could be a methyl (Me), an ammonium (NH_2), or a hydroxyl group.*